**REMARKS**

The Office Action and the cited and applied references have been carefully reviewed. No claim is allowed. Claims 1, 3-5, 10-21, 23-27, 33, 48, 49, and 53-116 presently appear in this application and define patentable subject matter warranting their allowance. Reconsideration and allowance are hereby respectfully solicited.

Amended claim 1 incorporates the features of claims 2 and 6 and is now limited to human hematopoietic $CD38^{-low}$ $CXCR4^{+}$ stem cells which are capable of migrating in response to SDF-1 and which have the capacity of migrating to, and of engraftment and repopulation of, the bone marrow in the host. This capacity is supported in the specification on page 5, lines 11-17.

The second capability in original claim 1 of adhering to stromal cells in response to an adhesion-inducing agent is now deleted from claim 1 but incorporated into new claim 53.

New claims 63 and 64 have been added to define the incubation time for the stimulation of the $CXCR4^{-/low}$ stems cells with cytokines. Claims 54-58 and 62-65 are parallel to these claims, but are dependent on claim 53. The time of incubation is a very unique characteristic of the present invention, as explained hereinafter when discussing Kanz et al (US 5,541,103), and is supported by the specification (page 6, lines 27-29; page

17, lines 20-21; page 34, lines 23-24; page 35, lines 23-27, etc.).

New claims 65-69 are dependent on claims 15 and 16 and define the features of the agents suitable for upregulating surface CXCR4 expression as defined previously in claims 12-14. New claims 72-79 are dependent on claim 33 and define the same features for the *in vitro* screening of the cells.

New claims 80-91 and 92-104 are two sets of claims defining the cells as pluripotent stem cells and not as a cell composition. These cells are supported by the specification, page 5, lines 15-20, that define the term "pluripotent stem cells" as "cells that have the capacity to migrate to the bone marrow of a transplanted recipient and to reconstitute the bone marrow and peripheral blood of said recipient with both myeloid and lymphoid lineages and also to proliferate without differentiating (self-renewal), said proliferation being measured by secondary transplantation in which the entire process is repeated in the second recipient".

Claims 104-116 define the cells as pluripotent stem cells obtained by the process of stimulation with a suitable agent to cause the expression of internalized CXCR4.

The expression "internalized CXCR4" used in claims 80-116 is supported by the specification, page 32, line 9. This is a well-known characteristic of the CXCR4 receptor. 472 citations

for the term "CXCR4 internalization" are identified just in a Google search.

Claims 7-9 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite. This rejection is obviated by the cancellation of claims 7-9.

Claims 1-14 have been rejected under 35 U.S.C. 102(a) as being anticipated by Mohle et al. (1991, Blood, 12: 4523-30). The examiner contends that Mohle teaches purified compositions of cells, including $CD34^-$ cells, $CD34^+$ cells, $CD34^+$ $CD38^{low}$ cells, $CD38^+$ cells, and $CD34^{low}$ cells, which express CXCR4 and migrate in response to SDF-1 (Mohle et al., pp. 4525-4527, and Figs. 1-3), and populations of cells wherein the majority of cells express CXCR4 and a subpopulation is $CXCR4^{-/low}$ (Mohle et al., p. 4526, Fig. 2), and thus takes the position that Mohle anticipates the presently claimed invention. This rejection is respectfully traversed.

Amended claim 1 and dependent claims therefrom now only define hematopoietic human $CD38^{-/low}$ $CXCR4^+$ stem cells that migrate in response to SDF-1 and have the capacity of migrating to, and of engraftment and repopulation of, the bone marrow in a host. These cells were obtained both from $CD38^{-/low}$ $CXCR4^+$ stem cells that express the CXCR4 receptor on the cell surface as well as from $CD38^{-/low}$ $CXCR4^{-/low}$ stem cells that have the potential to express CXCR4 on the cell surface and are converted to $CD38^-$

/low CXCR4+ cells upon stimulation with a suitable agent, this up-regulation of the CXCR4 receptor being an important feature of the invention. According to the present invention, human CXCR4+ stem cells have been obtained that were shown for the first time to be capable of engraftment and repopulation in animal models (see Examples 2-4, and Figs. 2-5).

Contrary to the present invention, Mohle discloses only progenitor cells and leukemic cells that express CXCR4 and migrate in response to SDF-1 (see title and throughout the paper). These cells do not, and have not been shown by Mohle, to have the capacity of migrating to, and of engraftment and repopulation of, the bone marrow in a host. Repopulation of the bone marrow is by definition a characteristic of stem cells. This is not an inherent capacity of cells which express CXCR4 and migrate in response to SDF-1. For example, in Fig. 3 of Mohle, it is shown that about 20-25% of the purified circulating CD34+ progenitor cells migrated in response to SDF-1, but as these are circulating cells, it means that there is no homing to the bone marrow and, as progenitor cells, they are not able to repopulate the bone marrow. It may be that the stem cells that remained in the 75-80% cells, which did not migrate in response to SDF-1, would migrate if the CXCR4 that underwent internalization would be expressed on the cell surface after

stimulation with a suitable agent as shown in the present application for the first time.

Accordingly, claim 1 as amended and claims dependent therefrom cannot be anticipated by Mohle. Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 15-27, 33, and 48-49 have been rejected under 35 U.S.C. 103 (a) as being unpatentable over Kanz et al. (U.S. Patent 5,541,103) in view of Mohle et al. The examiner refers to the methods of increasing the population of hematopoietic stem cells for use in clinical transplantation comprising up-regulating surface CXCR4 expression and sorting out those CXCR4 stem cells that migrate in response to SDF-1 (as defined in claim 15), to the methods wherein CXCR4 is up-regulated by stimulation with cytokines such as IL-6 and SCF, or stromal cells, or stromal cells and a mixture of IL-6 and SCF, and to the methods of screening for cells suitable for transplantation comprising the stimulation of cells with IL-6 and SCF followed by sorting of the cells for SDF-1 responsiveness.

According to the examiner, Kanz et al. teaches the preparation of hematopoietic stem cells useful for transplantation comprising stimulating cells with mixtures of cytokines including IL-6 and SCF-1 (columns 1 and 7-8). In particular, Kanz is said to teach that $CD34^+$ cells treated with

IL-6 and SCF-1 expand in culture and demonstrate increased colony forming potential which increases their usefulness for transplantation (columns 3-4). The examiner holds that Kanz teaches the stimulation of peripheral blood progenitor cells derived from cancer patients, and further suggests purifying the expanded peripheral blood progenitor cells from contaminating tumor cells (column 4, lines 19-31). The examiner acknowledges that Kanz does not specifically teach that the administration of IL-6 and SCF-1 results in increased expression of CXCR4 on the progenitor cells, but concludes that Kanz does teach the exact method steps recited by the claims for up-regulating surface CXCR4 expression and, thus, according to MPEP 222.01, a *prima facie* case of either anticipation or obviousness has been established. This rejection is respectfully traversed.

Kanz teaches the *ex vivo* expansion of peripheral blood progenitor cells of cancer patients by incubation of $CD34^+$ cells with cytokines for up to 28 days (Example 4) or for 21 days (Example 5), thus causing cell proliferation and producing a 20-fold up to 100-fold expansion (Column 8, lines 6-7).

Contrary to Kanz, the method of the present invention for up-regulating cell surface CXCR4 expression is not a cell expansion method. The method of the present invention does not alter the total number of cells but solely increases the number of cells in the cell population that expresses CXCR4 on the cell

surface. This is done by stimulation of the cells with an agent, namely a cytokine and/or at least one type of stromal cell that is involved in expansion of stem cells, but that according to the present invention are incubated with the cells for a relatively short period (up to 5 days, preferably for 1-2 days, and not for 3-4 weeks as disclosed by Kanz) and thus cause the expression of the internalized CXCR4 on the cell surface - there is no cell proliferation and the total number of the cell population is not increased.

The examiner combines the disclosures and teachings of Kanz and Mohle and states that while Kanz does not teach the sorting of cells that migrate in response to SDF-1, Mohle supplements Kanz and, thus, based on the motivation provided by Mohle for sorting $CXCR4^+$ progenitor cells which transmigrate in response to SDF-1 for use in transplantation in order to increase stem cell homing and migration, it would have been *prima facie* obvious to further purify the stem cell sproduced by Kanz by using the transmigration assay taught by Mohle.

As discussed above, both Mohle and Kanz use progenitor cells and not stem cells. Contrary to what the examiner wrote (page 7, last lines), Mohle did not show that the "$CXCR4^+$ hematopoietic progenitor cells which migrate in response to SDF-1 would have enhanced capability to migrate and home to the bone marrow which would increase their usefulness for

transplantation". This is a result that Mohle would wish to achieve, but which in reality was not achieved by Mohle. In addition, it is important to note that while progenitor cells may be used and are used in clinical transplantation, these cells will not cause repopulation of the bone marrow, a characteristic exhibited only by stem cells, and essential in severe clinical cases.

Accordingly, Kanz and Mohle cannot make obvious the presently claimed invention. Reconsideration and withdrawal of the rejection are therefore respectfully requested.

In view of the above, the claims comply with 35 U.S.C. §112 and define patentable subject matter warranting their allowance. Favorable consideration and early allowance are earnestly urged.

Respectfully submitted,

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